The Alcoholic Ferment of Yeast-juice. Part IV.—*The Fermentation of Glucose, Mannose, and Fructose by Yeast-juice.*

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The results previously communicated by the authors* were obtained exclusively with glucose, and in the present paper an account is given of the behaviour of mannose and fructose towards yeast-juice both in the presence and absence of added phosphate.

Buchner examined the fermentation of fructose by yeast-juice and found that it proceeded at precisely the same rate as that of glucose.† No experiments with mannose appear to have been previously performed.

The fructose employed throughout these experiments was Kahlbaum's crystallised fructose prepared from inulin. The mannose was prepared by the hydrolysis of ivory-nut and was purified by conversion into the phenylhydrazone, which was recrystallised from hot water and was finally decomposed by benzaldehyde in the usual manner.‡ All the experiments were performed at 25° in the presence of toluene.

I. *Relative Rates of Fermentation of Glucose, Mannose, and Fructose.*

Both mannose and fructose are freely fermented by yeast-juice, as they are also by living yeast. The relative rates of fermentation of these three sugars by yeast-juice vary somewhat in different experiments, but on the average of the experiments performed the fructose appears to be fermented rather more quickly than either mannose or glucose, whilst the mannose is also fermented slightly more rapidly than glucose.

Table I gives the experimental results, 25 c.c. of yeast-juice being employed in each case.

In experiments 1, 2, and 4 the rates were taken after the mixture had been incubated for about half an hour. In experiment 3, observations of rate were made during three different intervals, and the time during which the mixture had been incubated before the commencement of each of these was—(*a*) 25 minutes, (*b*) 70 minutes, (*c*) 130 minutes.

* 'Roy. Soc. Proc.,' B, 1906, vol. 77, p. 405 ; 1908, vol. 80, p. 299.

† 'Die Zymasegärung,' p. 100.

‡ Herzfeld, 'Ber. Deutsch. Chem. Ges.,' 1895, vol. 28, p. 440.

Table I.—Relative Rates of Fermentation of Glucose, Mannose, and Fructose by Yeast-juice.

No. of exp.	Amount of sugar.	Total volume.	Time in mins.	Rate of fermentation. Cubic centimetres evolved in the time given.			Ratio of rates.		
				Glucose.	Mannose.	Fructose.	Glucose.	Mannose.	Fructose.
1	1	25·6	45	3·2	3	5·3	1	0·94	1·66
2	1	25·6	60	24·8	28	29	1	1·13	1·17
3*a*	4	45	35	6·8	7·2	8·7	1	1·06	1·28
b	4	45	60	10·4	11	14·1	1	1·06	1·36
c	4	45	265	40·2	38·6	46·9	1	0·96	1·17
4	1·5	32·5	135	51·8	55·9	55·7	1	1·08	1·08

II. *Total Fermentation.*

The total weight of carbon dioxide evolved from an excess of the sugar by a given volume of yeast-juice was also found to be slightly greater with fructose than with glucose, whilst that evolved from mannose in the only two experiments made was considerably less than from glucose.

The following are the experimental results, 25 c.c. of yeast-juice being employed, and the incubation continued until fermentation had ceased:—

Table II.—Total Fermentation of Glucose, Mannose, and Fructose.

No. of exp.	Sugar added.	Total volume.	Total carbon dioxide in grammes.			Ratio of totals.		
			Glucose.	Mannose.	Fructose.	Glucose.	Mannose.	Fructose.
5	4	32·5	0·6556	0·4452	0·7436	1	0·68	1·13
6	4	32·5	0·7405	0·6226	0·8624	1	0·67	1·16

III. *Fermentation of Mannose by Yeast-juice in presence of Phosphate.*

Mannose behaves towards phosphates in the presence of yeast-juice in precisely the same manner as glucose.* A rapid rise in the rate of fermentation occurs; an extra amount of carbon dioxide and alcohol are produced which are equivalent to the phosphate added, and the phosphate is converted into a hexosephosphate which is not precipitable by magnesium citrate mixture and can be isolated in the form of a lead salt. As in the case of glucose, an optimum concentration of phosphate exists at which a maximum rate of fermentation occurs. Beyond this optimum, increase of concentration

* Harden and Young, *loc. cit.*

of phosphate lowers the rate of fermentation. The rates obtained with mannose and glucose in comparative experiments are approximately equal.

These phenomena are illustrated by the following experiments:—

Experiment 7.—Two quantities of 25 c.c. of yeast-juice + 5 c.c. of a solution containing 1 gramme of the sugar were incubated until a constant rate had been attained, and 5 c.c. of an approximately 0·3 molar solution of sodium phosphate were then added.

Experiment 8.—Two quantities of 25 c.c. of a different sample of yeast-juice + 1 gramme of the sugar were incubated as above and 10 c.c. of the same sodium phosphate solution (0·3 molar) were added.

The readings made after the addition of the phosphate are tabulated below, the numbers expressing the volume of carbon dioxide evolved in the five minutes preceding the time given in the first column:—

Table III.—Fermentation of Glucose and Mannose in presence of Phosphate.

Time after addition.	Carbon dioxide evolved in preceding 5 minutes.			
	Experiment 7.		Experiment 8.	
	Glucose.	Mannose.	Glucose.	Mannose.
5	4·4	6·4	22·4	22·5
10	8·2	6·0	23·8	26·7
15	9·1	6·8	22·2	22·1
20	9·6	7·0	16·8	14·5
25	7·9	6·0		
30	4·0	4·8		
35	1·5	2·7		
40	1·0	1·0		
45	1·1	1·0		

Experiment 9. *Formation of a Hexosephosphate.*—Twenty-five cubic centimetres of yeast-juice were incubated until the rate of fermentation became constant at 0·8 c.c. in five minutes. Ten cubic centimetres of a 0·3 molar solution of sodium phosphate were then added. The rate rose to 7·1 c.c. in five minutes, and incubation was continued until it had again fallen. The liquid was then boiled and filtered, and the amount of free phosphate estimated as $Mg_2P_2O_7$. The whole solution was found to yield 0·0567 gramme of $Mg_2P_2O_7$. The phosphate added corresponded to 0·3263 gramme $Mg_2P_2O_7$, and hence the difference between these quantities, $0·3263 - 0·0567 = 0·2696$, corresponds to the minimum amount of phosphate rendered non-precipitable by magnesium citrate mixture.

Experiment 10. *Equivalence of extra Carbon Dioxide evolved to the*

Phosphate added.—Parallel experiments with glucose, mannose, and fructose were made with yeast-juice from the same preparation, the method being that previously described.* Three quantities of 25 c.c. of yeast-juice + 5 c.c. of a solution containing 1 gramme of the sugar to be examined were incubated with toluene at 25° for one hour, and to each were then added 5 c.c. of a solution of sodium phosphate corresponding to 0·1632 gramme of $Mg_2P_2O_7$ and equivalent to 32·6 c.c. of carbon dioxide at N.T.P. The rates of fermentation were then observed until they had again fallen and attained a steady value, the gases being measured moist at 19°·3 and 760·15 mm.

Table IV.—Relation of Carbon Dioxide evolved to Phosphate added for Glucose, Mannose, and Fructose.

	Glucose.	Mannose.	Fructose.
Maximum attained	9·6	7	11·3
Final rate of juice, cubic centimetres in 5 minutes	1·1	0·96	1·08
Total evolved in 55 minutes after addition of phosphate	49·7	47·8	47·6
Correction for rate of juice in absence of phosphate	12·1	10·6	11·9
Total equivalent to phosphate	37·6	37·2	35·7
„ „ at N.T.P.	34·4	34·0	32·6

These numbers agree well with the value calculated from the phosphate added, viz., 32·6 c.c.

Experiment 11. *Effect of an Excess of Phosphate.*—Two quantities of 15 c.c. of yeast-juice + 7 c.c. of a solution of mannose containing 1 gramme of the sugar were treated with 10 and 15 c.c. respectively of a 0·6 molar solution of potassium phosphate, K_2HPO_4. The following readings were obtained, showing that in presence of 15 c.c. of the phosphate solution the rate is less than in presence of 10 c.c., and that in neither case is a high maximum rate attained:—

Table V.—Effect of an Excess of Phosphate on the Fermentation of Mannose.

Time after addition in minutes.	Rate in preceding 5 minutes.	
	10 c.c. phosphate.	15 c.c. phosphate.
10	2·2	2·1
20	1·9	1·6
30	2·4	1·4
40	3·0	1·5
60	4·2	1·7

* 'Roy. Soc. Proc.,' B, 1906, vol. 77, p. 414.

IV. *Fermentation of Fructose by Yeast-juice in the presence of Phosphate.*

Fructose, like mannose, agrees qualitatively with glucose in its behaviour towards phosphates, but it differs quantitatively from both these sugars in two important respects: (1) The optimum concentration of phosphate is much greater; (2) the maximum rate of fermentation attainable is much higher.

These points of resemblance and dissimilarity are brought out by the following experiments:—

(*a*) When a phosphate is added to yeast-juice containing fructose the rate of fermentation rises to a maximum and then falls to a rate which is usually slightly higher than the original rate of fermentation.

Experiment 12.—Ten cubic centimetres of a 0·3 molar solution of sodium phosphate were added to a mixture of 25 c.c. of yeast-juice and 1 gramme of fructose, the original rate of fermentation of which was 0·8 c.c. in five minutes. The total volume of the mixture was 35·6 c.c. The readings were as follows:—

Table VI.—Fermentation of Fructose in presence of Phosphate.

Time after addition of phosphate in minutes.	Cubic centimetre of CO_2 evolved in preceding 5 minutes.
5	14·9
10	21·3
15	21·7
20	17·3
25	10·9
30	2·0
35	1·5
40	1·0
45	1·2

The maximum rate attained varies very considerably with different samples of yeast-juice, as is shown by the following numbers (see Table VII), which refer in each case to 25 c.c. of yeast-juice.

It is interesting to note that the two high rates, 80 and 76·2 c.c. per five minutes, are equal to about half the rate obtainable with an amount of living yeast corresponding to 25 c.c. of yeast-juice, assuming that about 40 grammes of yeast are required to yield this amount of juice, and that this amount of yeast would give about 140 c.c. of carbon dioxide per five minutes at 25°, which has been found experimentally to be the average rate obtainable with the top yeast employed for these experiments.

Table VII.—Maxima attained by the Fermentation of Fructose in presence of Phosphate.

Number of experiment.	Volume of 0·6 molar phosphate solution added.	Total volume.	Maximum rate attained, cubic centimetres CO_2 in 5 minutes.
	c.c.	c.c.	
13	12·5	75	80
14	12·5	50	27·1
15	10	50	31·2
16	20	55	76·2

(*b*) Equivalence of the extra carbon dioxide evolved to the phosphate added. One example of this has already been given in Experiment 10, p. 338.

Experiment 17.—In another case the phosphate added was equivalent to 65·2 c.c. of CO_2 at N.T.P.

	c.c.
Gas evolved in 45 minutes	82·2
Correction for rate of juice 1·2 c.c. per 5 minutes for a period of 45 minutes	10·8
Gas evolved at 19 and 747·75	71·4
Volume at N.T.P.	64·2

(*c*) Production of a hexosephosphate non-precipitable by magnesium citrate mixture.

Experiment 18.—The experiment was carried out precisely as Experiment 9. The amounts of phosphate are expressed as $Mg_2P_2O_7$:—

Phosphate added	0·3263
Free phosphate after fermentation	0·0426
Phosphate rendered non-precipitable	0·2837

The solution after boiling was found to contain a hexosephosphate which has been isolated in the form of a lead salt and is at present undergoing investigation.

(*d*) Existence of an optimum concentration of phosphate.

The following table shows the maximum rates produced by the addition

of varying volumes of a 0·6 molar solution of potassium phosphate, K_2HPO_4, to yeast-juice and fructose. In all comparable experiments the total volumes were kept equal by the addition of a solution of potassium bicarbonate as previously explained for glucose;* in each case 2 grammes of fructose were employed. The maximum obtained and the optimum concentration are printed in thick type:—

Table VIII.—Maximum Rates of Fermentation and Optimum Concentrations of Phosphate for Fructose.

No. of experiment.	Volume of yeast-juice.	Total volume.	Cubic centimetres of 0·6 molar solution of K_2HPO_4 added.	Maximum rate per 5 minutes.
	c.c.	c.c.		c.c.
19*a*	5	25	0	0·5
b	5	25	**5**	**14·2**
c	5	25	10	5·5
d	5	25	15	1·8
20*a*	15	40	3	22·5
b	15	40	**7·5**	**25·4**
c	15	40	10	20·7
d	15	40	15	11·3
e	15	40	20	7·4
21*a*	10	35	3	31·5
b	10	35	**5**	**32·2**
c	10	35	7·5	28·5
d	10	35	10	20·2
e	10	35	15	9·2
f	10	35	20	5·7

It thus appears that, precisely as in the case of glucose, progressive increase in the concentration of phosphate beyond the optimum produces a corresponding decrease in the rate of fermentation, and at a high concentration the rate becomes extremely slow.

(*e*) Comparison of the optimum concentrations of phosphate and of the maximum rates produced at those concentrations for fructose and glucose.

The following results, which all refer to 10 c.c. of yeast-juice, clearly show that the optimum concentration of phosphate for the fermentation of fructose is from 1·5 to 10 times that for glucose, and that the maximum rate of fermentation for fructose is two to six times that of glucose.

* 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 307.

Table IX.—Optimum Concentrations of Phosphate and Maximum Rates of Fermentation for Fructose and Glucose.

No. of experiment.	Sugar, in grammes.	Total volume.	Optimum volume of 0·6 molar phosphate.		Maximum rate in cubic centimetres of CO_2 per 5 minutes.	
			Glucose.	Fructose.	Glucose.	Fructose.
22	2	35	2	5	7·5	32·2
23	4	50	1	10	5·4	28·4
24	1·6	23	2	5	8	17
25	1	25	1·75	5	5·2	25·9
26	2	25	5	7·5	16·2	31·2
27	2	20	2	3·5	7·9	22·6
28	2	22·5	0·75	2	3·4	22·2

V. *Effect of the Addition of Fructose on the Fermentation of Glucose or Mannose in presence of a large Excess of Phosphate.*

When the rate of fermentation of glucose or mannose by yeast-juice is greatly lowered by the presence of a large excess of phosphate, the addition of a relatively small amount of fructose causes rapid fermentation to occur. This induced activity is not due solely to the fermentation of the added fructose, since the amount of this sugar may be insufficient to yield the gas evolved.

The general nature of this phenomenon may be gathered from the following experiment:—

Experiment 29.—Two quantities of 25 c.c. of yeast-juice + 2 grammes glucose + 20 c.c. of 0·6 molar K_2HPO_4 solution + toluene were incubated at 25°. The amount of phosphate was largely in excess of the optimum, and the rate of fermentation was found to be 1·8 c.c. per five minutes.

A. To one of these were added 1 c.c. of glucose solution containing 0·2 gramme of the sugar, and 4 c.c. of the phosphate solution. The rate of fermentation fell to 1·5 and continued at this value.

B. To the other were added 1 c.c. of a solution containing 0·2 gramme of fructose and 4 c.c. of the phosphate solution. The rate of fermentation at once rose, as shown by the following readings.

As the evolution of carbon dioxide proceeds the phosphate is converted into hexosephosphate, and its effect on the fermentation of the glucose lessened, and hence in order to maintain the original concentration of phosphate it is necessary to add a fresh quantity at intervals to B, the amount required being calculated from the gas evolved, 13·5 c.c. of CO_2 at

Time after addition.	CO_2 evolved in preceding 5 minutes.	
	Substances added.	
	A. Glucose and phosphate.	B. Fructose and phosphate.
5 minutes	1·5	2·6
10 "	1·8	6·9
15 "	1·2	13·8
20 "	1·5	19·4
25 "	1·5	25·1

17°·2 and 762·4 mm. being equivalent to 2 c.c. of the phosphate solution. The further course of the experiment was as follows:—

B.

Time after addition of phosphate.	Gas evolved since last addition of phosphate.	Phosphate added 0·6 molar solution.	Rate per 5 minutes.
minutes.	c.c.	c.c.	c.c.
25	67·8	4	25·1
30	—	—	27·1
35	53·9	10	26·8
40	—	—	22·1
45	—	—	19·7
50	—	—	18·5
55	—	—	19·8
60	—	—	20·5
65	120·5	10	19·9
70	—	—	17·3
75	—	—	18·2
80	—	—	17·4
85	—	—	15·8
90	—	—	16·8
95	97·9	—	12·4
Total gas evolved	340·1		

Hence, although the concentration of the phosphate was never allowed to fall much below the original value and was generally considerably above it, the addition of 0·2 gramme of fructose to 2 grammes of glucose produced a total evolution of 340·1 c.c. of CO_2, corresponding to the total fermentation of 1·3 grammes of sugar (in the ratio $C_6H_{12}O_6 : 2\ CO_2$), whereas in the absence of fructose the total fermentation would have been only 28·5 c.c., corresponding to 0·22 gramme of sugar. As the fructose added was only 0·2 gramme and the subsequent evolution of carbon dioxide corresponded to

the total fermentation of 1·3 grammes of sugar, it is obvious that the addition of the fructose must have induced the fermentation of the glucose.

Experiment 30.—Similar results were obtained in another way by employing so large an excess of phosphate that the fermentation observed did not reduce the concentration of phosphate to the limit at which the rapid fermentation of glucose in the absence of added fructose became possible.

Four quantities of 15 c.c. of yeast-juice + 5 c.c. of a solution containing 2 grammes of glucose were employed—

1. 5 c.c. of 0·6 molar potassium phosphate solution were added.
2. 7·5 c.c. of phosphate solution were added.
3. 15 c.c. of phosphate solution were added.
4. 15 c.c. of phosphate solution and 0·5 c.c. of a solution containing 0·05 gramme of fructose were added.

The following observations were then made :—

Time.	Rate for 5 minutes.			
	1.	2.	3.	4.
5 minutes	2·0	1·9	0·8	4·2
10 „	2·6	1·6	1·1	4·0
15 „	3·7	2·2	0·6	6·6
20 „	3·7	2·1	0·8	8·9
25 „	5·2	2·6	0·7	11·6
30 „	6·4	2·9	0·6	14·2
35 „	9·6	3·5	0·7	14·8
Total evolved	33·2	16·8	5·3	64·3

In this case the amount of gas evolved in No. 4 is equivalent only to 4·8 c.c. of the phosphate solution, and the final concentration is therefore 10·2 c.c. of phosphate in 35 c.c. The concentration of phosphate, therefore, never falls as low as that present in No. 2 (7·5 in 27·5 or 9·5 in 35), and yet the fermentation is much more rapid than in this flask, which itself contains a concentration of phosphate greatly in excess of the optimum, as shown by a comparison with No. 1. The amount of carbon dioxide yielded by the complete fermentation of 0·05 gramme of fructose is only 13·5 c.c., so that there can be no doubt that most of the gas evolved was derived from the glucose.

(Note.—Experiments 1, 2, and 4 are not strictly comparable, since the contents of the flasks were not made up to the same volume, but the difference in rate due to this is negligible.)

VI. *Specific Character of the Inductive Action of Fructose.*

This inductive effect is specific to fructose and is not produced when glucose is added to mannose or fructose, or by mannose when added to glucose or fructose, under the proper conditions of concentration of phosphate in each case.

The experiments on this point were carried out by ascertaining in each case the excess of phosphate necessary to produce a slow rate of fermentation and then making two parallel experiments, one with, and the other without, the addition of the small quantity of the sugar to be tested for inductive power. All the observations were made with 15 c.c. of yeast-juice.

Table X.—Specific Character of the Inductive Effect of Fructose.

No. of experiment.	Cubic centimetres 0·6 molar phosphate.	Total volume.	Grammes of sugar present.			Gas evolved.	Time.
			Mannose.	Fructose.	Glucose.		
		c.c.				c.c.	mins.
31*a*	15	37	1	0	0	8·7	30
b	15	37	1	0·1	0	43·3	30
c	15	37	1	0	0·1	10·9	30
32*a*	10	32	1	0	0	18·9	30
b	10	32	1	0·1	0	76·3	30
33*a*	60	82·5	0	1	0	13·6	20
b	60	82·5	0·15	1	0	7·9	20
34*a*	10	30	0	0	1	11·4	30
b	10	30	0·1	0	1	14·1	30
35*a*	75	90	0	2	0	18·4	30
b	75	90	0	2	0·1	16·1	30

This remarkable property of fructose, taken in connection with the facts that this sugar in presence of phosphate is much more rapidly fermented than glucose or mannose, and that the optimum concentration of phosphate for fructose is much higher than for glucose or mannose, appears to indicate that fructose when added to yeast-juice does not merely act as a substance to be fermented, but, in addition, bears some specific relation to the fermenting complex.

All the phenomena observed are, indeed, consistent with the supposition that fructose actually forms a permanent part of the fermenting complex, and that, when the concentration of this sugar in the yeast-juice is increased, a greater quantity of the complex is formed. As the result of this increase in the

concentration of the active catalytic agent, the yeast-juice would be capable of bringing about the reaction with sugar in presence of phosphate at a higher rate, and at the same time the optimum concentration of phosphate would become greater, exactly as is observed. The question whether, as suggested above, fructose actually forms part of the fermenting complex, and the further questions, whether, if so, it is an essential constituent, or whether it can be replaced by glucose or mannose with formation of a less active complex, remain at present undecided, and cannot profitably be more fully discussed until the results of experiments now in progress are available.

It must, moreover, be remembered that different samples of yeast-juice vary to a considerable extent in their relative behaviour to glucose and fructose, so that the phenomena under discussion may be expected to vary with the nature and past history of the yeast employed.

Summary.

1. Mannose behaves towards yeast-juice both in the presence and in the absence of added phosphates in the same manner as glucose.

2. Fructose resembles both glucose and mannose in its behaviour towards yeast-juice, but in the presence of phosphates is much more rapidly fermented than the other sugars, and the optimum concentration of phosphate is much higher.

3. Fructose has the property of inducing rapid fermentation in presence of yeast-juice in solutions of glucose and mannose containing such an excess of phosphate that fermentation is only proceeding very slowly. No similar property is possessed by glucose or mannose.